Journal of Saudi Chemical Society (2015) **19**, 287–291

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

New phytoconstituents from the roots of *Aralia cachemirica* Decne

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Received 5 January 2012; accepted 26 March 2012
Available online 19 April 2012

KEYWORDS

Aralia cachemirica Decne;
Araliaceae;
Roots;
Fatty acid;
Diterpenes;
Sesterterpenes

Abstract *Aralia* species are used in traditional medicine to treat rheumatic arthritis, nephritis, lumbago, lameness, gastritis, inflammation and diabetes mellitus. Phytochemical investigation of the roots of *Aralia cachemirica* Decne (Araliaceae) collected from the Aharbal region of Kashmir afforded four new phytoconstituents identified as *n*-tetracont-19-enoic acid (**1**), 4α,4β,8β,10β,13β,17β-hexamethyl perhydrophenanthrenyl-3β-*n*-decanoate (**3**), tetrahydrocontinentalic acid (**4**) and 1β,4α,4β-trimethyl-6-(10,14,18-trimethyl-tridec-6-enyl)-cyclohexane-4β-ol (**5**) along with the known compounds continentalic acid (**2**), maltose (**6**) and sucrose (**7**). The structures of these phytoconstituents have been elucidated on the basis of spectral data analysis.

1. Introduction

Aralia cachemirica Decne (Araliaceae), known as Khoree and Banakhor, is a perennial aromatic lax shrub, up to 3 m tall, distributed in Afghanistan, Tibet, Kashmir, Himachal Pradesh, Uttarakhand and Sikkim (Pusalkar, 2009). *Aralia* species are used in traditional medicine to treat gastritis, rheumatic arthritis, inflammation, nephritis and diabetes mellitus (Oh et al., 2009; Liu et al., 2000). It is eaten by goats as a nutrient (Anon., 2003). *A. cachemirica* yielded aralosides (George et al., 1984), β-sitosterol (Anon., 2003), sugars (Bhat et al., 2010), essential oil (Shawl et al., 2009; Verma et al., 2010) and continentalic acid (Sharma et al., 2011). The plant showed hyperglycaemic (Bhat et al., 2005) and antibacterial (Sangwan et al., 2008) activities. This manuscript describes the isolation and characterization of new fatty acids, tetraterpinolide and diterpenic acid from the roots of *A. cachemirica*.

2. Materials and methods

2.1. General

The melting points were determined on a Perfit apparatus and are uncorrected. The IR spectra were recorded on KBr pellet using a jasco FT/IR-5000 instrument (FTS 135, Hongkong). The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were scanned on DRX-400 Avance 400 MHz spectrometer (Bruker-Biospin, Rheinstetten, Germany) using $CDCl_3$ as solvent and TMS as internal standard. FAB-MS were

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.jscs.2012.03.004

measured using JEOL-JMS-DX 303 spectrometer (Peabody, MA, USA). Column ($450 \times 4 \times 0.2$ cm) chromatography was performed on silica gel (60–120 mesh, Qualigens, Mumbai, India) and thin layer chromatography on silica gel G-coated TLC plates (Merck). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying reagents.

2.2. Plant material

The dried roots of *A. cachemirica* were collected from the Aharbal region of Kashmir and was authenticated by Dr. A.R. Naqshi, the Department of Taxonomy, University of Kashmir, Srinagar with a voucher specimen No. PS/UKS/AR-1 and was deposited in herbarium for future reference in the Department of Pharmaceutical Sciences, University of Kashmir, Srinagar.

2.3. Extraction and isolation

The air dried roots (2.5 kg) were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with methanol for 72 h. The methanolic extract was concentrated under reduced pressure to obtain a dark brown viscous mass (210 g). Small portion of extract was analyzed chemically to determine the presence of different chemical constituents. The concentrated extract (150 g) was dissolved in methanol (250 ml) and adsorbed on silica gel (60–120 mesh) for column chromatography. The slurry was air dried and chromatographed over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, and 1:3), pure chloroform and finally the mixture of chloroform and methanol (99:1, 97:3, 19:1, 23:2, 9:1, 3:1, and 1:1, 1:3). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated:

2.4. n-Tetracont-19-enoic acid (*1*)

Elution of the column with petroleum ether gave colourless amorphous powder of **1**, recrystallized from acetone–methanol (1:1), yield 0.36%, m.p. 54–55 °C, R_f 0.3 (petroleum ether–chloroform, 9:1); IR ν_{max} (KBr): 3300, 2950, 2843, 1690, 1643, 1410, 1315, 1280, 1130, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.03 (1H, m, $w_{1/2}$ = 9.8 Hz, H-19), 5.00 (1H, m, $w_{1/2}$ = 9.8 Hz, H-20), 2.14 (2H, t, J = 7.1 Hz, H_2-2), 2.01 (2H, m, H_2-18), 1.98 (2H, m, H_2-21), 1.52 (4H, m, 2×CH_2), 1.29 (62H, brs, 31×CH_2), 0.91 (3H, t, J = 6.5 Hz, Me-40); ^{13}C NMR ($CDCl_3$): δ 180.06 (C-1), 122.31 (C-19), 115.02 (C-20), 55.20 (C-2), 40.17 (CH_2), 39.52 (CH_2), 37.61 (CH_2), 35.42 (CH_2), 31.87 (CH_2), 28.12 (CH_2), 29.45 (28×CH_2), 22.56 (CH_2), 18.51 (Me-40); +ve FAB MS m/z (rel. int): 591 $[M+H]^+$ ($C_{40}H_{79}O_2$) (12.3), 283 (9.8), 307 (19.5).

2.5. Continentalic acid (*2*)

Elution of the column with petroleum ether–chloroform (9:1) afforded colourless crystals of **2**, recrystallized from methanol, yield 1.36%; m.p. 159–160 °C, IR ν_{max} (KBr): 3280, 1693, cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.55 (1H, brs, H-14), 5.73 (1H, dd, J = 9.9 Hz, 7.2 Hz, H-15), 5.14 (1H, brs, H_2-16a), 4.92 (2H, d, J = 12.6 Hz, H_2-16b), 1.25 (3H, brs, Me-19), 1.01 (3H, brs, Me-20), 0.65 (3H, brs, Me-17); ^{13}C NMR ($CDCl_3$): δ 38.51 (C-1), 19.22 (C-2), 36.40 (C-3), 44.04 (C-4), 56.09 (C-5), 29.21 (C-6), 35.78 (C-7), 137.93 (C-8), 50.51 (C-9), 37.90 (C-10), 29.71 (C-11), 29.35 (C-12), 39.15 (C-13), 127.96 (C-14), 147.14 (C-15), 112.91 (C-16), 24.08 (C-17), 184.83 (C-18), 19.56 (C-19), 13.79 (C-20); +ve FAB MS m/z (rel. int): 303 $[M+H]^+$ ($C_{20}H_{31}O_2$) (11.2).

2.6. Cachemiridiol (*3*)

Elution of column with petroleum ether–chloroform (9:1) afforded colourless crystals of **3**, recrystallized from acetone–methanol (1:1), yield 0.36%, R_f 0.4 (petroleum ether–benzene, 17:3), m.p. 85–86 °C, IR ν_{max} (KBr): 3410, 2925, 2872, 1725, 1642, 1430, 1210, 1145, 1052 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.26 (1H, d, J = 5.6, H-6), 5.07 (1H, m, H-11), 4.25 (1H, dd, J = 5.5, 9.6 Hz, H-3α), 3.25 (2H, t, J = 9.5 Hz, H_2-19), 2.53 (2H, t, J = 7.2 Hz, H_2-2′), 2.27 (2H, m, H_2-7), 2.18 (2H, m, H_2-12), 1.58 (2H, m, H_2-2), 1.98 (1H, m, H-14), 1.85 (2H, m, H_2-1), 1.71 (1H, m, H-13), 1.68 (1H, m, H-17), 1.63 (2H, m, H_2-15), 1.58 (2H, m, CH_2), 1.54 (4H, m, 2×CH_2), 1.42 (16H, brs, 8×CH_2), 1.19 (3H, brs, Me-22), 1.02 (3H, d, J = 6.2 Hz, Me-24), 0.95 (3H, d, J = 6.5 Hz, Me-25), 0.88 (3H, brs, Me-20), 0.83 (3H, t, J = 6.1 Hz, Me-10′), 0.80 (3H, brs, Me-21), 0.68 (3H, brs, Me-23); ^{13}C NMR ($CDCl_3$): δ 45.07 (C-1), 34.18 (C-2), 69.94 (C-3), 42.01 (C-4), 140.92 (C-5), 120.22 (C-6), 39.22 (C-7), 56.28 (C-8), 137.82 (C-9), 38.36 (C-10), 127.46 (C-11), 38.96 (C-12), 49.52 (C-13), 55.28 (C-14), 35.28 (C-15), 33.48 (C-16), 50.52 (C-17), 33.28 (C-18), 61.52 (C-19), 25.35 (C-20), 20.46 (C-21), 18.95 (C-22), 18.65 (C-23), 20.48 (C-24), 21.19 (C-25), 172.12 (C-1′), 31.24 (C-2′), 28.96 (C-3′), 28.96 (C-4′), 28.96 (C-5′), 28.96 (C-6′), 28.52 (C-7′), 28.52 (C-8′), 22.34 (C-9′), 14.21 (C-10′); +ve FAB MS m/z (rel. int): 529 $[M+H]^+$ ($C_{35}H_{61}O_3$) (9.3), 373 (22.15), 155 (12.5).

2.7. Tetrahydrocontinentic acid (*4*)

Elution of column with chloroform–methanol (49:1) afforded colourless amorphous powder of **4**, recrystallized from acetone–methanol (1:1), yield 1.26%, R_f 0.5 (chloroform–ethyl acetate, 4:1), m.p. 115–116 °C, IR ν_{max} (KBr): 3410, 2926, 2850, 1697, 1539, 1470, 1370, 1250, 1120 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.16 (1H, m, H-5β), 1.98 (2H, m, H_2-1), 1.86 (2H, m, H_2-2), 1.79 (2H, m, H_2-3), 1.71 (2H, m, H_2-14), 1.68 (2H, m, H_2-7), 1.53 (1H, m, H-9), 1.47 (1H, m, H-8), 1.35 (2H, m, H_2-6), 1.32 (3H, brs, Me-19), 1.29 (2H, m, H_2-12), 1.23 (2H, m, H_2-11), 1.21 (2H, m, H_2-15), 1.16 (3H, brs, Me-20), 1.14 (3H, brs, Me-17), 0.85 (3H, t, J = 6.3 Hz, Me-16); ^{13}C NMR ($CDCl_3$): δ 41.20 (C-1), 23.60 (C-2), 39.35 (C-3), 47.51 (C-4), 56.81 (C-5), 28.73 (C-6), 37.25 (C-7), 55.05 (C-8), 55.86 (C-9), 38.73 (C-10), 25.96 (C-11), 36.60 (C-12), 42.50 (C-13), 44.31 (C-14), 21.37 (C-15), 14.77 (C-16), 28.16 (C-17), 179.05 (C-18), 18.25 (C-19), 17.39 (C-20); +ve FAB MS m/z (rel. int): 307 $[M+H]^+$ ($C_{20}H_{35}O_2$) (18.3).

2.8. Araliasesterterpenol (*5*)

Elution of column with chloroform–methanol (19:5) afforded colourless crystals of **5**, recrystallized from chloroform–methanol (1:1), yield 0.15%, R_f 0.25 (toluene–ethyl acetate, 4:1), m.p. 218–

219 °C, IR ν_{max} (Kbr): 3350, 2955, 2845, 1640, 1470, 1265, 1080 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.13 (1H, m, H-7), 3.52 (1H, dd, J = 5.1, 8.5 Hz, H-4α), 2.50 (1H, m, H-1), 2.26 (2H, m, H_2-8), 2.18 (2H, m, H_2-3), 2.01 (2H, m, H_2-2), 1.77 (2H, m, H_2-3), 1.71 (1H, m, H-10), 1.62 (1H, m, H-14), 1.58 (1H, m, H-18), 1.52 (6H, brs, H_2-9, H_2-11, H_2-12), 1.25 (8H, brs, H_2-13, H_2-15, H_2-16, H_2-17), 1.23 (3H, brs, Me-21), 1.21 (3H, brs, Me-22), 1.11 (3H, d, J = 6.1 Hz, Me-20), 0.96 (3H, d, J = 6.3 Hz, Me-23), 0.93 (3H, d, J = 6.0 Hz, Me-24), 0.88 (3H, d, J = 6.5 Hz, Me-19), 0.83 (3H, d, J = 6.1 Hz, Me-25); ^{13}C NMR ($CDCl_3$): δ 42.63 (C-1), 13.95 (C-2), 13.76 (C-3), 71.08 (C-4), 55.16 (C-5), 140.63 (C-6), 127.13 (C-7), 41.36 (C-8), 31.15 (C-9), 36.38 (C-10), 30.88 (C-11), 29.16 (C-12), 29.12 (C-13), 37.46 (C-14), 28.43 (C-15), 28.37 (C-16), 28.57 (C-17), 33.39 (C-18), 17.63 (C-19), 21.89 (C-20), 27.16 (C-21), 24.09 (C-22), 21.63 (C-23), 15.02 (C-24), 18.56 (C-25); +ve FAB MS m/z (rel. int): 365 $[M+H]^+$ ($C_{25}H_{49}O$) (5.5).

2.9. Maltose (6)

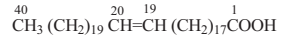
Elution of the column with chloroform–methanol (9:1) gave colourless crystals of **6**, yield 0.12%, recrystallized from aqueous alcohol, m.p. 102–103 °C, R_f 0.40 (*n*-butanol–acetone–pyridine–water, 10:10:5:5; v/v); +ve FAB MS m/z (rel. int): 343 $[M+H]^+$ ($C_{12}H_{23}O_{11}$) (1.7).

2.10. Sucrose (7)

Further elution of the column with chloroform–methanol (9:1) yielded colourless crystals of **7**, yield 0.36%, recrystallized from methanol, m.p. 161–164 °C, R_f 0.20 (*n*-butanol–acetone–pyridine–water, 10:10:5:5; v/v); +ve FAB MS m/z (rel. int): 343 $[M+H]^+$ ($C_{12}H_{23}O_{11}$) (2.1).

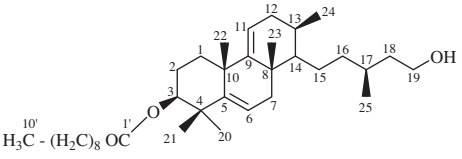
3. Results and discussion

Compound **1** was obtained as a colourless amorphous powder from petroleum ether eluents. It yielded effervescence with sodium bicarbonate and showed characteristic IR absorption bands for carboxylic group (3300, 1690 cm^{-1}), unsaturation (1643 cm^{-1}) and long aliphatic chain (721 cm^{-1}). Its mass spectrum showed a molecular ion peak at m/z 591 $[M+H]^+$ consistent with the molecular formula of an unsaturated fatty acid $C_{40}H_{79}O_2$. The ion fragments generating at m/z 307 $[CH_3(CH_2)_{19}CH{=}CH]^+$ and 283 [M-307; $(CH_2)_{17}COOH]^+$ suggested the location of the vinylic linkage at C-19. The ^1H NMR spectrum displayed two one-proton multiplets at δ 5.03 and 5.00 with half-width of 9.8 Hz each assigned to *cis*-oriented vinylic H-19 and H-20 protons, respectively, a two-proton triplet at δ 2.14 (J = 7.1 Hz) ascribed to methylene H_2-2 protons adjacent to the carboxylic function, other methylene protons from δ 2.01 to 1.29 and a three-proton triplet at δ 0.91 (J = 6.5 Hz) accounted for the terminal C-40 primary methyl protons. The ^{13}C NMR spectrum of **1** exhibited signals for the carboxylic carbon at δ 180.06 (C-1), vinylic carbons at δ 122.31 (C-19) and 115.02 (C-20), methylene carbons between δ 55.20 and 22.56 and methyl carbon at δ 18.51 (C-40). The absence of any signal between δ 5.00 and 2.14 in the ^1H NMR spectrum and between δ 115.02 and 22.56 in the ^{13}C NMR spectrum ruled out any carbinol carbon in the molecule. The ^1H–^1H COSY spectrum of **1** showed a correlation of H-19 with H-20, H_2-18 and H_2-21. On the basis of these evidences the structure of **1** has been characterized as *n*-tetracont-19-enoic acid. This is a new fatty acid.



1

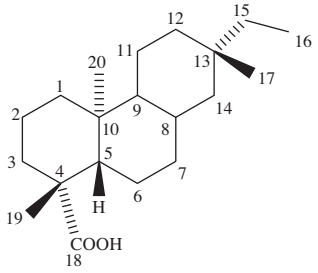
Compound **3**, named cachemiridiol, was obtained as a colourless crystalline mass from petroleum ether–chloroform (4:1) eluents. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3410 cm^{-1}), ester function (1725 cm^{-1}) and unsaturation (1642 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **3** was determined at m/z 529 $[M+H]^+$ consistent with the molecular formula of a sesterterpenic ester, $C_{35}H_{61}O_3$. The ion peaks arising at m/z 155 $[CH_3(CH_2)_8CO]^+$ and 373 [M-155; $C_{25}H_{43}O_2]^+$ indicated that *n*-capric acid was esterified with a tricyclic sesterterpenoid. The ^1H NMR spectrum of **6** exhibited a one-proton doublet at δ 5.26 (J = 6.5 Hz) and a one-proton multiplet at δ 5.07 that were assigned to vinylic H-6 and H-11, respectively. A one-proton doublet at δ 4.25 (J = 5.5, 9.6 Hz) and a two-proton triplet at δ 3.25 (J = 9.5 Hz) were ascribed to the oxygenated methine H-3α and hydroxymethylene H_2-19 protons, respectively. Four three-proton broad singlets at δ 1.19, 0.88, 0.80 and 0.68, two three-proton doublets at δ 1.02 (J = 6.2 Hz) and 0.95 (J = 6.5 Hz) and a three-proton triplet at δ 0.83 (J = 6.1 Hz) were attributed correspondingly to the tertiary methyl C-22, C-20, C-21 and C-23, secondary methyl C-24 and C-25 and primary methyl C-10′ protons, all attached to saturated carbons. The other methine and methylene protons resonated from δ 2.53 to 1.24. The ^{13}C NMR spectrum of **3** displayed signals for ester carbon at δ 172.12 (C-1′), vinylic carbons at δ 140.92 (C-5), 120.22 (C-6), 137.82 (C-9) and 127.46 (C-11), oxygenated methine carbons at δ 69.94 (C-3), hydroxymethylene carbon at δ 61.52 (C-19) and methyl carbons from δ 25.35 to 14.21. The ^1H–^1H COSY spectrum showed correlations of H-3 with H_2-1, H_2-2 and Me-20; H-6 with H_2-7 and Me-21; H-11 with H_2-12 and H-13; and H_2-19 with H-17 and H_2-18. The HMBC spectrum of **3** exhibited interactions of C-5 with Me-20, Me-21, H-6 and H_2-7; C-9 with Me-23, Me-22, H-11 and H_2-12; C-1′ with H-3 and H_2-2′; and H_2-19 with H_2-18 and H-17. On the basis of these evidences the structure of **3** has been characterized as 4α,4β,8β,10β,13β,17β-hexamethyl perhydrophenanthrenyl-3β-*n*-decanoate. This is a new tricyclic sesterterpenic ester.



3

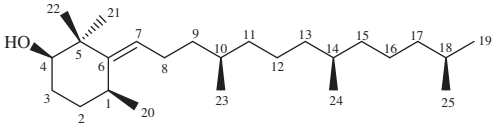
Compound **4**, named tetrahydrocontinentalic acid, was obtained as a colourless amorphous powder from chloroform–methanol (49:1) eluents. It produced effervescence with sodium bicarbonate solution and showed IR absorption bands for carboxylic group (3410, 1697 cm^{-1}). On the basis of mass

and ^{13}C NMR spectra the molecular ion peak of **4** was established at m/z 307$[M+H]^+$ corresponding to the molecular formula of a tricylcic diterpenic acid $C_{20}H_{35}O_2$. The ^1H NMR spectrum of **4** exhibited three broad singlets at δ 1.32, 1.16 and 1.14 and a triplet at δ 0.85 (J = 6.3 Hz), all integrating with three protons each, ascribed to the tertiary C-19, C-20 and C-17 and primary C-16 methyl protons, respectively. The methine and methylene protons appeared between δ 2.16 and 1.21. The ^{13}C NMR spectrum of **4** displayed signals for the carboxylic carbons at δ 179.05 (C-18), methyl carbons at δ 14.77 (C-16), 28.16 (C-17), 18.29 (C-19) and 17.39 (C-20) and methylene and methine carbons between δ 56.81 and 21.37. The absence of any signal beyond δ 2.16 in the ^1H NMR spectrum and between δ 179.05 and 56.81 in the ^{13}C NMR spectrum suggested the saturated nature of the molecule and ruled out the existence of any carbinol carbon. The ^1H–^1H COSY spectrum of **4** showed correlations of Me-19 with H_2-3 and H-5; Me-20 with H_2-1, H-9 and H-8; and Me-17 with H_2-12, H-8, H_2-14, H-15 and Me-16. The HMBC spectrum of **4** exhibited interactions of C-18 with H_2-3, H-5 and Me-19; and C-13 with H_2-12, H_2-14, Me-17, H_2-15 and Me-16′. On the basis of the foregoing account the structure of **4** has been elucidated as tetrahydrocontinentalic acid. This is a new diterpenic acid.



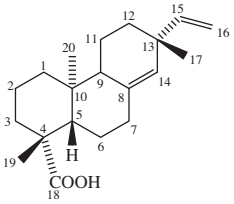
4

Compound **5**, designated as araliasesterterpenol, was obtained as a colourless crystalline mass from chloroform–methanol (19:5) eluents. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3350 cm^{-1}) and unsaturation (1640 cm^{-1}). On the basis of FAB mass and ^{13}C NMR spectra, the molecular ion peak of **5** was determined at m/z 365 $[M+H]^+$ corresponding to the molecular formula of a monocyclic unsaturated sesterterpenol, $C_{25}H_{49}O$. The ^1H NMR spectrum of **5** exhibited a one-proton multiplet at δ 5.13 that was assigned to vinylic H-7. A one-proton doublet at δ 3.52 (J = 5.1, 8.5 Hz) was ascribed to the α-oriented carbinol H-4 proton. Two three-proton broad singlets at δ 1.23 and 1.21 were attributed to the tertiary C-21 and C-22 methyl protons, respectively. Five three-proton doublets at δ 1.11 (J = 6.1 Hz), 0.96 (J = 6.3 Hz), 0.93 (J = 6.0 Hz), 0.88 (J = 6.5 Hz) and 0.83 (J = 6.1 Hz) were accounted for the secondary C-20, C-23, C-24, C-19 and C-25 methyl protons, respectively. The remaining methine and methylene protons appeared from δ 2.50 to 1.25. The ^{13}C NMR spectrum of **5** displayed important signals for vinylic carbons at δ 140.63 (C-6) and 127.13 (C-7), carbinol carbon at δ 71.08 (C-4) and methyl carbons between δ 27.16 and 15.02. The ^1H–^1H COSY spectrum of **5** showed correlations of H-4 with H_2-2, H_2-3 and Me-21; H-7 with H_2-8, H_2-9, H-1, Me-20 and Me-22; and H-18 with H_2-17, Me-19 and Me-25. The HMBC spectrum of **5** exhibited interactions of C-4 with H_2-3, H_2-2 and Me-21; C-6 with H-1, Me-20, Me-21, Me-22, H-7 and H_2-8; and C-18 with H_2-17, H_2-16, Me-19 and Me-25. On the basis of these evidences the structure of **5** was formulated as 1β,4α,4β-trimethyl-6-(10,14,18-trimethyltridec-6-enyl) cyclohexan-4β-ol. This is a new monocyclic sesterterpenol.



5

Compounds **2**, **6** and **7** are the known phytoconstituents that were identified as continentalic acid (Sharma et al., 2011), maltose and sucrose, respectively.



2

4. Conclusion

The present work has characterized several chemical compounds from the roots of *A. cachemirica*. The existing knowledge regarding its phytoconstituents may be increased by the present phytochemical investigation which may be responsible for medicinal property of the plant and may be used as chromatographic marker of the drug.

Acknowledgement

The authors are thankful to the Head, Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute, Lucknow, for recording mass spectra of the compounds.

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